The microbiome-gut-brain axis

Timothy G. Dinan[1,2], and John F. Cryan[1,3]

[1] APC Microbiome Institute, University College Cork, Cork, Ireland
[2]Department of Psychiatry and Neurobehavioural Science, University College Cork, Cork, Ireland
[3]Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

Corresponding author: Prof. Ted Dinan, Department of Psychiatry, University College Cork, Ireland. t.dinan@ucc.ie

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Abstract/Summary

Gut microbes are capable of producing most neurotransmitters found in the human brain. While these neurotransmitters primarily act locally in the gut, modulating the enteric nervous system, evidence is now accumulating to support the view that gut microbes through multiple mechanisms can influence central neurochemistry and behavior. This has been described as a fundamental paradigm shift in neuroscience. Bifidobacteria for example can produce and increase plasma levels of the serotonin precursor tryptophan, which is fundamental in regulating mood, appetite and gastrointestinal function. Certain Lactobacilli have been shown to produce gamma-aminobutyric acid (GABA) and to alter brain GABA receptor expression and behavior. IBS is regarded as the prototypic disorder of the brain-gut-microbiota axis which can be responsive to probiotic therapy. Recently, the concept of a psychobiotic has been introduced in the literature. A psychobiotic is a bacteria which when ingested in adequate amounts can have a positive mental health benefit. Translational studies indicate that certain bacteria may impact upon stress responses and cognitive functioning. Manipulating the gut microbiota with psychobiotics, prebiotics or even antibiotics offers a novel approach to altering brain function and treating gut-brain axis disorders such as depression and autism.

Key Points

- Gut microbes can communicate with the brain through a variety of routes including the vagus nerve, short chain fatty acids, cytokines and tryptophan
- Psychobiotics are bacteria which when ingested in adequate amounts produce a positive mental health benefit.
- The brain-gut-microbiota axis represents a paradigm shift in neuroscience and provides a novel target for treating not just IBS but conditions such as depression, autism and Parkinson's disease.

Introduction

The human adult gut contains over 1kg of bacteria, essentially the same weight as the human brain[1]. It is generally estimated that the gut is inhabited by 10^{13}-10^{14} micro-organisms, which is more than ten times the number of human cells in our bodies and contains over 100 times as many genes as in our genome[2]. Amazingly, the genomic and biochemical complexity of the microbiota exceeds that of the brain. Studies of the brain-gut-microbiota axis have been described as a paradigm shift in neuroscience [3]. Increasing evidence points to the fact that appropriate diversity in the gut microbiota is not only essential for gut health but also for normal physiological functioning in other organs and especially the brain. An altered gut microbiota in the form of dysbiosis at the extremes of life, both in the neonate and in the elderly, can have a profound impact on brain function. Such a dysbiosis might emerge for a variety of reasons including the mode of birth delivery, diet, antibiotic and other drug exposure. Given the fact that the brain is dependent on gut microbes for essential metabolic products it is not surprising that a dysbiosis can have serious negative consequences for brain function both from a neurological and mental health perspective. While much of the early data emerged from animal studies, mainly rodent based, there are now an increasing number of human studies translating the animal findings.

In this review we will focus upon the routes of communication between the gut and brain, examine a prototypic disorder of the brain gut axis, explore the ways in which gut dysbiosis may evolve and provide an up-to-date account of behavioral and neurological pathologies associated with dysbiosis.

Brain-gut-microbiota communication

The brain-gut-microbiota axis is a bidirectional communication system enabling gut microbes to communicate with the brain, and the brain with the gut [4]. While brain-gut communication has been a subject of investigation for decades an exploration of gut microbes within this context has only featured in recent years. The mechanisms of signal transmission are complex and not fully elucidated, but include neural, endocrine, immune, and metabolic pathways [5,6]. Preclinical studies have implicated the vagus nerve as a key route of neural communication between microbes of the gut and centrally-mediated behavioural effects, as demonstrated by the elimination of central *Lactobacillus rhamnosus* effects following vagotomy[7] and the fact that humans who have underwent vagotomy at an early age have a decreased risk of certain neurological disorders[8]. The gut microbiota also regulates key central neurotransmitters such as serotonin by altering levels of precursors; for example *Bifidobacterium infantis* has been shown to elevate plasma tryptophan levels and thus influence central 5-HT transmission[9]. Intriguingly, synthesis and release of neurotransmitters from bacteria has been reported; *Lactobacillus* and *Bifidobacterium* species can produce gamma-aminobutyric acid (GABA): *Escheridia*, *Bacillus* and *Saccharomyces spp*. can produce noradrenaline: *Candida, Streptococcus, Escheridia and Enterococcus spp.* can produce serotonin: *Bacillus* can produce dopamine: and *Lactobacillus* can produce acetylcholine [10,11]. These microbially synthesised neurotransmitters can cross the mucosal layer of the intestines, though it is highly unlikely that they directly influence brain function. Even if they enter the blood stream, which is by no means certain, they are incapable of crossing the blood brain barrier (BBB). Their impact on brain function is likely to be indirect acting on the enteric

nervous system. Short chain fatty acids (SCFAs) which include butyrate, propionate and acetate are essential metabolic products of gut microbial activity and may exert central effects either through G-protein coupled receptors, though such receptors are sparsely concentrated in the brain. It is more likely that they act as epigenetic modulators through histone deacetylases (HDACs)[2]. SCFAs are also involved in energy balance and metabolism, and can modulate adipose tissue, liver tissue and skeletal muscle and function [12]. Immune signalling from gut to brain mediated by cytokine molecules is another documented route of communication [13]. Cytokines produced at the level of the gut can travel via the bloodstream to the brain. Under normal physiological circumstances it is unlikely that they cross the BBB, but increasing evidence indicates a capacity to signal across the BBB and to influence brain areas such as the hypothalamus where the BBB is deficient. It is through the latter mechanism the cytokines interleukin (IL)-1 and IL-6 activate the hypothalamic-pituitary-adrenal axis (HPA), bringing about the release of cortisol. This is the most potent activator of the stress system.

The HPA which provides the core regulation of the stress response can significantly impact the brain-gut-microbiota axis [14-20]. It is increasingly clear and probably of relevance in a number of pathological conditions that psychological or physical stress can significantly dysregulate the HPA and subsequently the brain-gut-microbiota axis, for example in IBS[21].

---Insert Fig. 1 here---

Multiple lines of approach have been used to interrogate the brain-gut-microbiota axis especially in animal model systems; these include the use of germ-free animals, potential probiotic agents, antibiotics, animals exposed to pathogens and the use of stress to

determine the effects of dysregulating the axis. The largest naturalistic study of a gut pathogen and the impact on the brain-gut axis was as a result of the Walkerton catastrophe. The contamination of the Walkerton water supply occurred in 2000 claimed seven lives and left over two thousand people ill. The *E. coli* outbreak was caused by farm runoff contaminating the town's water supply. Those infected had significant risk of developing post-infective-IBS and many had co-morbid depression/anxiety[22]. To a greater extent than any prior study this natural disaster provided clear cut support for the notion of post-infective IBS.

Brain-gut-microbiota axis and extremes of life

The intestinal microbiota of newborn infants is characterized by low diversity and a relative dominance of the phyla *Proteobacteria* and *Actinobacteria* in the early post-natal period, a time at which there is enormous brain development. With the passage of time, the microbiota becomes more diverse with the emergence and dominance of *Firmicutes* and *Bacteroidetes* [23-25]. Full-term, vaginally delivered babies born to healthy mothers who are breast fed and non-antibiotic treated have an optimal development of the neonatal microbiota [26]. The characteristic intestinal microbiota observed in healthy full-term infants is disturbed in preterm infants [27], who are frequently delivered by caesarean section, receive antibiotics and may have problems feeding [28]. Furthermore, preterm infants possess a functionally immature gut with low levels of acidity in the stomach, due to insufficient gastric acid secretion and their requirement for more frequent feeding [28-30]. These events lead to an increase in the prevalence of potentially pathogenic bacteria in the GI tract and less microbial diversity than full term infants [31-33]. The extent to which these features play a role in the development of cerebral palsy and subsequent autism are

the subject of research and ongoing debate [34]. What is clear is that complex brain maturation and the increasing sophistication of the gut microbiota are highly correlated. To date many of our assumptions are based on correlational data from which we cannot conclusively conclude a causative impact.

When elderly people in nursing homes are compared with those in the community large scale differences are detected. Those in nursing homes have a far less diverse microbiota and this has been attributed to a less varied diet[35]. However, it is possible that pathological factors that lead to admission into nursing homes such as deteriorating cognitive function and less physical activity might play an important role in the decreased microbial richness and not the less diverse diet. On-going studies should clarify this issue and there is a challenge for the food industry to produce diets for the elderly which will help to sustain microbial diversity.

What is abundantly clear is that a dyregulated gut microbiota either in early childhood or in an aging population significantly increases the likelihood of brain dysfunction. The precise relationship between these observations is far from understood. Determining the mechanisms and pathways underlying microbiota-brain interactions may yield novel insights into individual variations and perhaps enable the development of new treatments for a range of neurodevelopmental and neurodegenerative disorders, ranging from autism to Parkinson's disease.

IBS as prototype

IBS is the prototypic disorder of the brain-gut-microbiota axis, generally perceived as a having a biopsychosocial aetiology[36] and frequently co-morbid with depression or

anxiety.. The most important single risk factors are female gender, younger age and preceding gastrointestinal infections. Recent studies suggest that trauma in childhood especially sexual abuse may be an important risk factor[37]. The aspect of dysbiosis in IBS is important and will be dealt with elsewhere, but aspects of gut to brain communication are clearly altered. For example elevated levels of plasma pro-inflammatory cytokines are found and there is an exaggerated pituitary-adrenal response to corticotropin-releasing hormone, together with augmented visceral pain responses. A recent study found that fasting serum levels of SCFAs did not differ between patients with IBS and controls [38]. However, the postprandial levels of total SCFAs, acetic acid, propionic acid, and butyric acid were found to be significantly lower in patients with IBS compared with healthy controls. An epigenetic model of IBS has been proposed [36] which is consistent with the potential epigenetic modulating effects of butyrate, the levels of which are altered substantially in the post-prandial state.

Treatments for IBS which do not take into account this complex pathophysiology are likely to be of very limited benefit.

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Depression

IBS and depression are frequently co-morbid and the latter is associated with the presence of biomarkers of inflammation such as elevated interleukin IL-6, tumor necrosis factor alpha (TNFα) and the acute phase protein, C reactive protein (CRP)(45). Similar elevated biomarkers of inflammation have been seen in anxiety states and are known to occur as a result of stress. The site at which these pro-inflammatory molecules is

produced in depression is not known and it has yet to be determined whether the elevation is core to the pathophysiology or merely epiphenomenal. There is evidence from rodent studies to indicate that stress alters the gut barrier function allowing lipopolysaccharide (LPS) and other molecules to gain access to the bloodstream stimulating toll-like receptor 4 (TLR4) and other TLRs resulting in the production of inflammatory cytokines (46). If this does occur in depression, which has yet to be definitively demonstrated, it would explain the pro-inflammatory phenotype observed. The McMaster group using germ-free and specific pathogen-free mice, demonstrated that the early life stress of maternal separation alters the HPA and colonic cholinergic neural regulation in a microbiota-independent fashion [39]. However, they showed that the microbiota is required for the induction of anxiety-like behaviour and behavioural despair. Colonization of adult germ-free maternally separated and control mice with the same microbiota produces distinct microbial profiles, which are associated with altered behaviour in maternally separated, but not in control mice. The results suggest that maternal separation-induced changes in host physiology lead to intestinal dysbiosis, which is a critical determinant of the abnormal behaviour that characterizes this model of early-life stress. Prior studies in maternally separated rats demonstrated an altered behavioural phenotype when these animals reached maturity and also decreased diversity in the microbiota [20]. Does this decreased diversity translate to patients with major depression?

In a recent study the faecal microbiota was sequenced[40]. Forty-six patients with depression and 30 healthy controls were recruited. . High-throughput pyrosequencing

showed that, according to the Shannon index, increased fecal bacterial alpha-diversity was found in those currently depressed but not in a group who had responded to treatment . Bacteroidetes, Proteobacteria, and Actinobacteria were increased, whereas Firmicutes was significantly reduced. Despite the profound inter-individual variability, levels of several predominant genera were significantly different between the depressives and controls. Most notably, the depressives had increased levels of Enterobacteriaceae and Alistipes but reduced levels of Faecalibacterium. The authors conclude that further studies are necessary to elucidate the temporal and causal relationships between gut microbiota and depression and to evaluate the suitability of the microbiome as a biomarker. When rats are given a humanised microbiota from depressed patients as opposed to healthy controls they develop a depressive phenotype from a behavioural and immune perspective.

Autism

Autism is a neurodevelopmental disorder whose prevalence is apparently on the increase. It is characterised by a failure of language acquisition and a lack of sociability. It is frequently associated with GI symptoms[41] the relevance of which has been a longstanding source of controversy. Up to 70% of patients with the syndrome report abdominal symptoms and hence the view that it is a disorder of the brain-gut axis. Our group at the APC Microbiome Institute examined the behaviour of mice raised in a germ-free environment [42,43]. The mice were tested in a three chamber facility, where a germ-free mouse was placed in the middle chamber with a familiar mouse in one chamber and a novel mouse in the third. The germ-free mouse spent as much time with the familiar as

with the novel mouse; this is in contrast to the behaviour of conventionally colonised mice who spend more time with the novel than the familiar mouse. Germ-free mice are also more likely to spend time with an object than with another mouse, a decidedly abnormal behaviour for a sociable animal. Colonisation of the germ free mice does partially normalise their behaviour patterns. These behavioural changes are associated with significant alterations in underlying neurochemistry.

Work from the late Paul Patterson & Sarkis Mazmanian's group in an animal model demonstrated that the microbiota modulates behavioural and physiological abnormalities associated with neurodevelopmental disorders such as autism [44]. They used the maternal immune activation model induced by poly-IC injection during pregnancy and found altered gastrointestinal barrier defects and microbiota alterations. Oral treatment with the human commensal *Bacteroides fragilis* was shown to correct gut permeability and most interestingly stereotyped and other abnormal behaviours. Furthermore, a metabolite found in the abnormal animals was observed to transfer the phenotype to naïve animals and to be reduced by *Bacteroides fragilis.*

Increasing attention is currently being paid to oxytocin the hypothalamic peptide which has been shown to increase sociability. The oxytocin receptor knockout mouse shows considerable deficits in social behaviour [45] and some small scale preliminary studies in humans indicate that intra-nasally administered oxytocin may positively alter social behaviour patterns. A few large clinical trials are under way to test oxytocin and related therapies for autism spectrum disorder [46]. There is still considerable debate as to whether or not the preclinical findings translate to the clinical setting and if they do which patients and which aspects of the syndrome are likely to benefit most. Intriguingly, a recent study

indicates that a probiotic bacteria can influence hypothalamic posterior pituitary activity and increase oxytocin levels raising the possibility of influencing social behaviour by targeting the gut microbiota [47].

The faecal microbiota in patients with autism spectrum disorder has been sequenced [48]. In the most recently published study Tomova et al examined the microbiota in Slovakian children. The faecal microbiota of autistic children showed a significant decrease of the Bacteroidetes/Firmicutes ratio and elevation of the amount of Lactobacillus spp. There was a modest elevation in Desulfovibrio spp and a correlation with the severity of autism. A probiotic diet normalised the Bacteroidetes/Firmicutes ratio and Desulfovibrio spp levels. As recently summarised by Mayer & colleagues there is a paucity of large comprehensive studies of the microbiome in autism [3]. Again the issue of chicken or egg emerges; are these changes induced by stereotyped diets seen in many individuals as a product of obsessional behaviour patterns? Also the heterogeneous nature of the disease needs to be taken into account and much more effort is needed to tease out the exact role of the microbiome in both the aetiology and treatment of the disorder.

Parkinson's disease

In marked contrast to autism Parkinson's disease tends to be diagnosed generally in old age; it is the second most common neurodegenerative disorder and affects 1-2% of the population over 65 years of age. It is a movement disorder characterised by degeneration of the zona compacta neurons of the substantia nigra. The most common GI symptoms are constipation, appetite loss, weight loss, dysphagia, sialorrhea and gastro-oesophageal reflux[49]. Alpha-synuclein-aggregates, the major neuropathological marker in Parkinson's

disease, are present in the submucosal and myenteric plexuses of the enteric nervous system, prior to their detection in the brain, which may indicate a gut to brain "prion-like" spread [50].

The gut microbiota has been sequenced in patients with Parkinson's disease[51]. On average, the abundance of Prevotellaceae in the feces of Parkinson's disease patients was reduced by almost 80% compared with controls. A logistic regression analysis based on the abundance of four bacterial families and the severity of constipation identified Parkinson's disease patients with 66.7% sensitivity and 90.3% specificity. The relative abundance of Enterobacteriaceae was highly correlated with the severity of postural instability and gait difficulty. The findings suggest that the intestinal microbiome is altered in Parkinson's disease and is related to motor phenotype. Large prospective studies beginning in the early stages of the disorder are required.

It has been suggested that microbiota transplantation might benefit patients with Parkinson's disease but there is as yet no conclusive evidence[52]. Neither are there any reports of controlled trials of probiotics/psychotiotics.

Psychobiotics

Psychobiotics were first defined as the family of probiotics that, ingested in appropriate quantities, had a positive mental health benefit[53]. Recently, the definition has been expanded to include prebiotics, which are dietary, soluble fibres for example galactooligosaccharides (GOS) or fructooligosaccharides (FOS) that stimulate the growth of intrinsic commensal microbiota. There is now an enormous volume of preclinical data to support the concept of psychobiotics. Understandably, clinical data is less abundant

but nonetheless is emerging. Given the demonstrated efficacy of probiotics in IBS[54] and the high co-morbidity between IBS and stress related mental health issues such as anxiety and depression it is not surprising that certain probiotics might positively impact on mental health.

Tillisch et al [55] administered healthy female participants either a placebo or a mixture of probiotics (*Bifidobacterium animalis Lactis, Streptococcus thermophiles, Lactobacillus bulgaricus*, and *Lactococcus lactis Lactis*), which were consumed over four weeks. Participants underwent functional magnetic resonance imaging (*f*MRI) to determine how probiotic ingestion affected neuropsychological activity. During image acquisition, participants were shown emotional faces that are known to capture attention and cause brain activation. Relative to placebo, probiotic-treated participants showed decreased activity in a functional network associated with emotional, somatosensory, and interceptive processing, including the somatosensory cortex, the insula, and the periaqueductal gray. In marked contrast, placebo participants showed increased activity in these regions in response to emotional faces. This is interpreted as evidence of a probiotic-induced reduction in network-level neural reactivity to negative emotional information.

A recent prebiotic study carried out in Oxford found a significant impact on stress responses [56]. Healthy male and female participates consumed either BGOS, FOS, or a placebo. In comparison to the other two groups, participants who consumed BGOS showed significantly reduced waking-cortisol responses, which are a robust marker of anxiety, stress, and depression risk [57]. Furthermore, participants completed an emotional dot-probe task measuring vigilance, or attention to negative stimuli, which is also a

marker of anxiety and depression. Participants taking BGOS showed substantially attenuated vigilance on this task, suggesting reduced attention and reactivity to negative emotions. Overall, the data support the view that the specific prebiotic has anxiolytic activity.

Takada et al [58] examined the effects of *Lactobacillus casei* strain Shirota (LcS) on gut-brain interactions under stressful conditions. A double-blind, placebo-controlled trials were conducted to examine the effects of LcS on psychological and physiological stress responses in healthy medical students whilst undergoing examination stress. Subjects received LcS-fermented milk or placebo daily for 8 weeks prior to taking an examination. Subjective anxiety scores, salivary cortisol, and the presence of physical symptoms were analysed. In a parallel animal study, rats were fed a diet wither with or without LcS for 2 weeks, then submitted to water avoidance stress (WAS). Plasma corticosterone concentration and the expression of cFos and corticotropin releasing factor (CRF) in the paraventricular nucleus (PVN) were measured immediately after WAS. Academic stress resulted in increases in salivary cortisol and an increase in physical symptoms, both of which were significantly suppressed in the LcS group. In rats pretreated with LcS, WAS-induced increases in plasma corticosterone were significantly suppressed, and the number of CRF-expressing cells in the PVN was reduced. Intriguingly, intragastric administration of LcS was found to stimulate gastric vagal afferent activity in a dose-dependent manner. The results suggest that LcS may positively impact stress responses by acting through the vagus nerve. In a study of university students we have found that a *Bifidobacterium longum* decreased morning waking cortisol levels, reduced subjective levels of anxiety

and modestly improved aspects of cognitive functioning, an effect that was associated with altered EEG activity.

A large scale cross-sectional study has examined the impact of probiotics on measures of social anxiety[59]. Seven hundred and ten young adults completed self-report measures of fermented food consumption, neuroticism, and social anxiety. An interaction model, controlling for demographics, general consumption of healthful foods, and exercise frequency, showed that exercise, neuroticism, and fermented food consumption significantly and independently predicted social anxiety. Furthermore, fermented food consumption also interacted with neuroticism in predicting social anxiety. For those with high neuroticism scores, a high frequency of fermented food consumption resulted in fewer symptoms of social anxiety. The data suggest that fermented foods containing probiotics may have a protective effect against social anxiety symptoms for those at higher genetic risk, as assayed by trait neuroticism.

Sternbergen et al [60] tested a multispecies probiotic containing *Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus salivarius*, and *Lactococcus lactis* in non-depressed individuals using a triple-blind, placebo-controlled, randomized, design, Twenty healthy participants received a 4-week probiotic food-supplement intervention with the multispecies probiotics, while 20 control participants received an inert placebo for the same period. Subjects who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood. The results provide evidence that probiotics may help reduce negative thoughts associated with sad mood.

Romijn and Rucklidge in their systematic review [61] add a note of caution to the above optimistic findings concluding that more trials are necessary before any definitive inferences can be made about the efficacy of probiotics in mental health applications. Further studies of a translational nature are certainly required.

Summary/Discussion

The role of the microbiota-gut-brain access in the genesis of IBS symptoms is now largely accepted, though several questions remain unanswered. How does stress, especially early life stress dysregulate the axis? Can IBS subtypes be delineated on the basis of the microbiota? If patients with IBS have co-morbid psychiatric illness does the latter resolve if the former is treated with probiotics?

We have an enormous number of pre-clinical studies implicating the gut microbiota in other stress-related conditions and in disorders at the extremes of life. Far more translational studies are required. The human studies to date support the view that the gut microbiota is altered in major depression and that psychobiotics, either in the form of prebiotics or probiotics van impact anxiety and depressive symptoms in healthy subjects. We have no clear indication of efficacy in diseased populations. In the neurodevelopmental disorder autism, which is usually diagnosed in early childhood, GI symptoms are common and an altered microbiota has been reported, while at the other end of the developmental spectrum old age related frailty correlates with decreased gut microbial diversity. Whether fecal microbiota transplantation is an appropriate therapeutic option in at least some brain-gut axis disorders remains to be determined.

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References

1. **Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. *Journal of psychiatric research.* Apr 2015;63:1-9.**
2. **Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. *Genes, brain, and behavior.* Jan 2014;13(1):69-86.**
3. **Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. Gut microbes and the brain: paradigm shift in neuroscience. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Nov 12 2014;34(46):15490-15496.**
4. **Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature reviews. Gastroenterology & hepatology.* May 2009;6(5):306-314.**
5. **El Aidy S, Dinan TG, Cryan JF. Gut Microbiota: The Conductor in the Orchestra of Immune-Neuroendocrine Communication. *Clinical therapeutics.* May 1 2015;37(5):954-967.**
6. **Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. *Frontiers in physiology.* 2011;2:94.**
7. **Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America.* Sep 20 2011;108(38):16050-16055.**
8. **Svensson E, Horvath-Puho E, Thomsen RW, et al. Vagotomy and subsequent risk of Parkinson's disease. *Annals of neurology.* Oct 2015;78(4):522-529.**
9. **Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience.* Nov 10 2010;170(4):1179-1188.**
10. **Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS pathogens.* Nov 2013;9(11):e1003726.**
11. **Lyte M. Microbial endocrinology and the microbiota-gut-brain axis. *Advances in experimental medicine and biology.* 2014;817:3-24.**
12. **Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature reviews. Endocrinology.* Oct 2015;11(10):577-591.**
13. **El Aidy S, Dinan TG, Cryan JF. Immune modulation of the brain-gut-microbe axis. *Frontiers in microbiology.* 2014;5:146.**
14. **Wang Y, Kasper LH. The role of microbiome in central nervous system disorders. *Brain, behavior, and immunity.* May 2014;38:1-12.**
15. **Tillisch K. The effects of gut microbiota on CNS function in humans. *Gut microbes.* May-Jun 2014;5(3):404-410.**

16. **Scott LV, Clarke G, Dinan TG. The brain-gut axis: a target for treating stress-related disorders. *Modern trends in pharmacopsychiatry.* 2013;28:90-99.**
17. **Moloney RD, Desbonnet L, Clarke G, Dinan TG, Cryan JF. The microbiome: stress, health and disease. *Mammalian genome : official journal of the International Mammalian Genome Society.* Feb 2014;25(1-2):49-74.**
18. **O'Mahony SM, Clarke G, Dinan TG, Cryan JF. Early-life adversity and brain development: Is the microbiome a missing piece of the puzzle? *Neuroscience.* Oct 1 2015.**
19. **O'Mahony SM, Hyland NP, Dinan TG, Cryan JF. Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology.* Mar 2011;214(1):71-88.**
20. **O'Mahony SM, Marchesi JR, Scully P, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological psychiatry.* Feb 1 2009;65(3):263-267.**
21. **Dinan TG, Quigley EM, Ahmed SM, et al. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology.* Feb 2006;130(2):304-311.**
22. **Marshall JK, Thabane M, Garg AX, et al. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. *Gut.* May 2010;59(5):605-611.**
23. **Backhed F. Programming of Host Metabolism by the Gut Microbiota. *Ann. Nutr. Metab.* 2011;58:44-52.**
24. **Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science.* Jun 10 2005;308(5728):1635-1638.**
25. **Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* Mar 4 2010;464(7285):59-U70.**
26. **Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics.* Aug 2006;118(2):511-521.**
27. **Dennison B. Definition of preterm delivery. *Br. Med. J.* 1976;2(6049):1449-1449.**
28. **Hoy CM, Wood CM, Hawkey PM, Puntis JWL. Duodenal microflora in very-low-birth-weight neonates and relation to necrotizing enterocolitis. *J. Clin. Microbiol.* Dec 2000;38(12):4539-4547.**
29. **Sondheimer JM, Clark DA. Gastric pH in healthy preterm infants - effect of age and feeding type. *Gastroenterology.* 1985 1985;88(5):1593-1593.**
30. **Sondheimer JM, Clark DA, Gervaise EP. Continuous gastric pH measurement in young and older healthy preterm infants receiving formula and clear liquid feedings. *J. Pediatr. Gastroenterol. Nutr.* 1985 1985;4(3):352-355.**
31. **Arboleya S, Binetti A, Salazar N, et al. Establishment and development of intestinal microbiota in preterm neonates. *FEMS Microbiol. Ecol.* Mar 2012;79(3):763-772.**
32. **Chang JY, Shin SM, Chun J, Lee J-H, Seo J-K. Pyrosequencing-based Molecular Monitoring of the Intestinal Bacterial Colonization in Preterm Infants. *J. Pediatr. Gastroenterol. Nutr.* Nov 2011;53(5):512-519.**

33. Jacquot A, Neveu D, Aujoulat F, et al. Dynamics and Clinical Evolution of Bacterial Gut Microflora in Extremely Premature Patients. ***Journal of Pediatrics.*** **Mar 2011;158(3):390-396.**

34. Mangiola F, Ianiro G, Franceschi F, Fagiuoli S, Gasbarrini G, Gasbarrini A. Gut microbiota in autism and mood disorders. ***World journal of gastroenterology : WJG.*** **Jan 7 2016;22(1):361-368.**

35. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. ***Nature.*** **Aug 9 2012;488(7410):178-184.**

36. Dinan TG, Cryan J, Shanahan F, Keeling PW, Quigley EM. IBS: An epigenetic perspective. ***Nature reviews. Gastroenterology & hepatology.*** **Aug 2010;7(8):465-471.**

37. Park SH, Videlock EJ, Shih W, Presson AP, Mayer EA, Chang L. Adverse childhood experiences are associated with irritable bowel syndrome and gastrointestinal symptom severity. ***Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.*** **Apr 8 2016.**

38. Undseth R, Jakobsdottir G, Nyman M, Berstad A, Valeur J. Low serum levels of short-chain fatty acids after lactulose ingestion may indicate impaired colonic fermentation in patients with irritable bowel syndrome. ***Clinical and experimental gastroenterology.*** **2015;8:303-308.**

39. De Palma G, Blennerhassett P, Lu J, et al. Microbiota and host determinants of behavioural phenotype in maternally separated mice. ***Nature communications.*** **2015;6:7735.**

40. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. ***Brain, behavior, and immunity.*** **Aug 2015;48:186-194.**

41. Li Q, Zhou JM. The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder. ***Neuroscience.*** **Jun 2 2016;324:131-139.**

42. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. ***Molecular psychiatry.*** **Feb 2014;19(2):146-148.**

43. Borre YE, Moloney RD, Clarke G, Dinan TG, Cryan JF. The impact of microbiota on brain and behavior: mechanisms & therapeutic potential. ***Advances in experimental medicine and biology.*** **2014;817:373-403.**

44. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. ***Cell.*** **Dec 19 2013;155(7):1451-1463.**

45. Chini B, Leonzino M, Braida D, Sala M. Learning about oxytocin: pharmacologic and behavioral issues. ***Biological psychiatry.*** **Sep 1 2014;76(5):360-366.**

46. Shen H. Neuroscience: The hard science of oxytocin. ***Nature.*** **Jun 25 2015;522(7557):410-412.**

47. Erdman SE, Poutahidis T. Probiotic 'glow of health': it's more than skin deep. ***Beneficial microbes.*** **Jun 1 2014;5(2):109-119.**

48. Tomova A, Husarova V, Lakatosova S, et al. Gastrointestinal microbiota in children with autism in Slovakia. *Physiology & behavior.* Jan 2015;138:179-187.
49. Park H, Lee JY, Shin CM, Kim JM, Kim TJ, Kim JW. Characterization of gastrointestinal disorders in patients with parkinsonian syndromes. *Parkinsonism & related disorders.* May 2015;21(5):455-460.
50. Felice VD, Quigley EM, Sullivan AM, O'Keeffe GW, O'Mahony SM. Microbiota-gut-brain signalling in Parkinson's disease: Implications for non-motor symptoms. *Parkinsonism & related disorders.* Jun 2016;27:1-8.
51. Scheperjans F, Aho V, Pereira PA, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement disorders : official journal of the Movement Disorder Society.* Mar 2015;30(3):350-358.
52. Dinan TG, Cryan JF. The impact of gut microbiota on brain and behaviour: implications for psychiatry. *Current opinion in clinical nutrition and metabolic care.* Nov 2015;18(6):552-558.
53. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. *Biological psychiatry.* Nov 15 2013;74(10):720-726.
54. Didari T, Mozaffari S, Nikfar S, Abdollahi M. Effectiveness of probiotics in irritable bowel syndrome: Updated systematic review with meta-analysis. *World journal of gastroenterology : WJG.* Mar 14 2015;21(10):3072-3084.
55. Tillisch K, Labus J, Kilpatrick L, et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology.* Jun 2013;144(7):1394-1401, 1401 e1391-1394.
56. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology.* May 2015;232(10):1793-1801.
57. Bhagwagar Z, Hafizi S, Cowen PJ. Increased salivary cortisol after waking in depression. *Psychopharmacology.* Oct 2005;182(1):54-57.
58. Takada M, Nishida K, Kataoka-Kato A, et al. Probiotic Lactobacillus casei strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* Feb 20 2016.
59. Hilimire MR, DeVylder JE, Forestell CA. Fermented foods, neuroticism, and social anxiety: An interaction model. *Psychiatry research.* Aug 15 2015;228(2):203-208.
60. Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, behavior, and immunity.* Aug 2015;48:258-264.
61. Romijn AR, Rucklidge JJ. Systematic review of evidence to support the theory of psychobiotics. *Nutrition reviews.* Oct 2015;73(10):675-693.

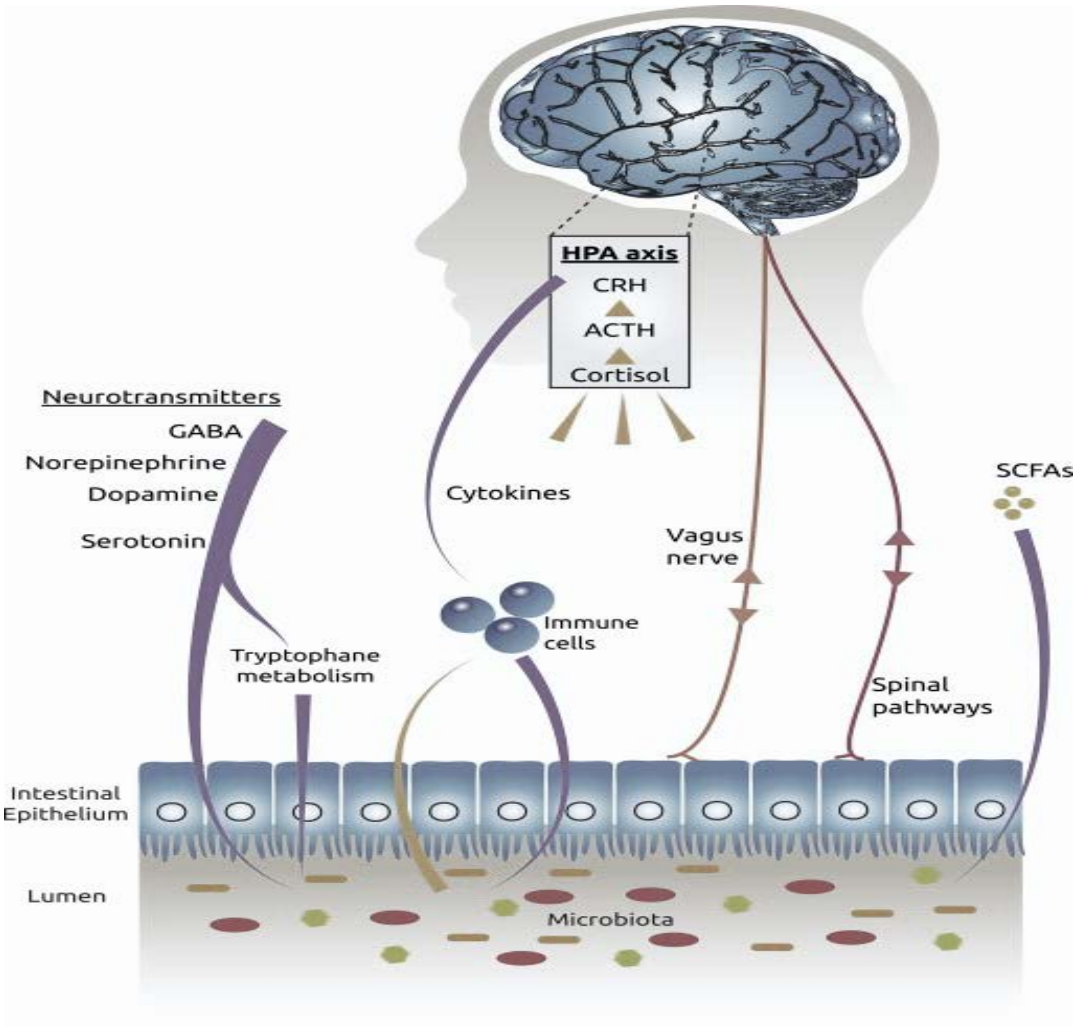
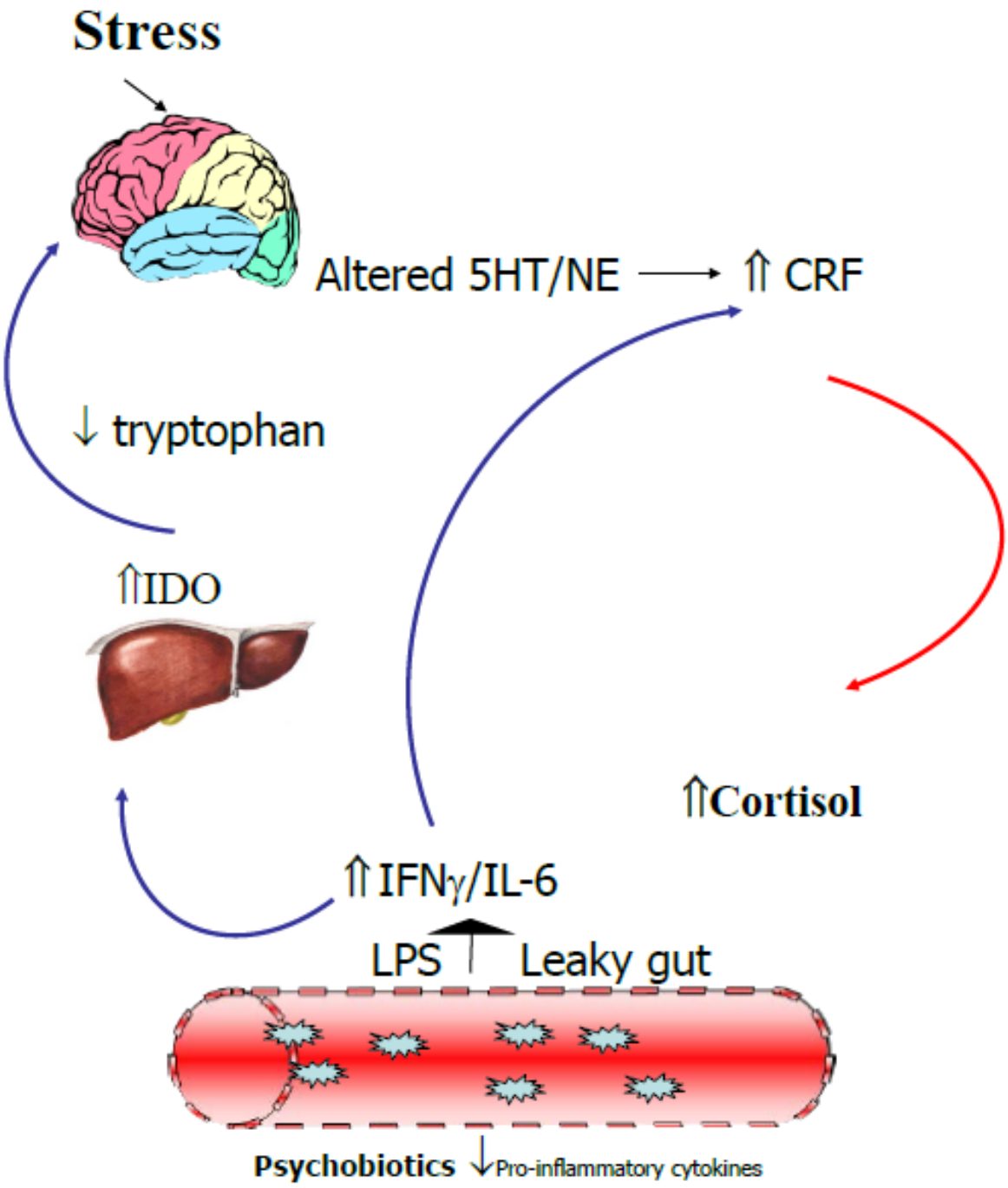


Fig 1 Routes of communication between gut microbes and brain. These include the vagus nerve, short chain fatty acids (butyrate, propionate, acetate), cytokines and tryptophan

Fig 2 Model of irritable bowel syndrome. Psychological stress or infection leads to activation of the hypothalamic-pituitary-adrenal-axis (HPA) with elevation in cortisol and also changes in gut permeability. Lipopolysaccharide (LPS) enters the bloodstream increasing pro-inflammatory cytokines and in turn altering tryptophan metabolism. In turn this leads to alterations in 5HT and glutamate neurotransmission. Psychobiotics may impact by decreasing gut permeability and signaling the brain via the vagus nerve and other routes.


Stress
Altered 5HT/NE ⟶ ⇑ CRF
↓ tryptophan
⇑IDO
⇑Cortisol
⇑ IFNγ/IL-6
LPS
Leaky gut
Psychobiotics ↓Pro-inflammatory cytokines